

AERIAL RESPIRATION IN THE LONGJAW MUDSUCKER *GILlichthys mirabilis* (TELEOSTEI: GOBIIDAE)

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Fishes that normally inhabit poorly oxygenated waters are often endowed with some mechanism enabling them to respire aerially (Carter, 1931, 1957; Bertin, 1958; Saxena, 1963). Bertin classified such adaptations as: (1) arborescent and labyrinthiform branchial organs, (2) pharyngeal organs, including the buccopharyngeal cavity and its diverticula, (3) esophageal organs, including the swimbladder and lungs, and (4) intestinal organs. Most reported air-breathers are fresh-water species, usually inhabitants of stagnant tropical swamps where deoxygenation is widespread in the protected, shaded waters. Marine fishes, which inhabit exposed waters subject to continuous wind mixing and oxygenation, generally lack special air-breathing organs (Carter, 1957), although Saxena (1963) recognized the "fishes of the estuaries" as constituting a "main association" of air-breathing species. The Gobiidae and related Blenniidae comprise one of the few groups of primarily marine teleosts that reportedly contain several species with such organs (*cf.* Schöttle, 1931; Ogliastro, 1947; Bertin, 1958). These species, moreover, often inhabit marginal areas such as mangrove swamps or quiet estuaries where characteristically large spatial, diurnal, and seasonal fluctuations of oxygen, depth, *etc.* create for them problems shared with denizens of fresh-water swamps.

The longjaw mudsucker (or simply "mudsucker") *Gillichthys mirabilis* Cooper is one of several gobies that can breathe either aerially or aquatically as the occasion requires. Like other air-breathing gobies, its accessory respiratory organ is buccopharyngeal of Bertin's type 2: at the surface the fish gulps air, which is then held in the highly vascularized buccopharynx for respiratory exchange. Typically it inhabits the shallow muddy backwaters and tidal flats from central and southern California, including the Salton Sea, southward along the Pacific Coast of Baja California to the Gulf of California, where in the northern half it is sympatric with its only congener, *G. seta* (Ginsburg) (Barlow, 1961b, 1963). At low tide *G. mirabilis* lives in small pools or even "... above the water level in burrows in the bank" (Barlow, 1961b). In an enclosed, constant-level saline lagoon on the U.C.S.B. campus the fish is continuously submerged but often tolerates almost anoxic conditions. Although it occurs naturally in waters of moderate to high salinity, it can tolerate even soft fresh water for varying periods up to two weeks (Barlow, 1961b; personal communication). Therefore, because its natural environment is unstable, *Gillichthys* must periodically tolerate fluctuating oxygen concentrations, near-anoxic conditions, and emergence.

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Similar adaptations in other gobies, such as the mudskipper *Periophthalmus*, have been carefully described (*e.g.*, Schöttle, 1931). Little has been reported, however, of the specific adaptation of *Gillichthys* to aerial respiration, although Barlow (1961a, 1961b, 1963) thoroughly investigated its taxonomy and geographical variation, both morphological and physiological, and Weisel (1947) as well as Barlow (1961a, 1961b) briefly noted its accessory ability to respire aerially. In the present study, therefore, we shall describe the environmental aspects of its aerial respiration by: (1) analyzing its behavioral adaptation in deoxygenated water of gulping at the surface and retaining air bubbles; (2) demonstrating respiratory gaseous exchange in these bubbles while they are retained; (3) describing the structure of the repository of these bubbles (buccopharyngeal organ), including its morphological adjustment before an episode of gulping; and (4) relating this adjustment to a possible accessory function of the swimbladder.

MATERIALS AND METHODS

Caught locally in an enclosed lagoon and in a tidal slough, the fish were held in well-aerated running sea water of salinity 33.1–34.0‰ at 16–20° C. in 15-gallon wooden aquariums. At 20° C., dissolved oxygen measured about 7 mg./l., carbon dioxide 2–3 p.p.m. In the lagoon, however, oxygen ranged from 0 mg./l. to supersaturation, while carbon dioxide was commonly 16–25 p.p.m. Experimental fish were studied in controlled oxygen environments maintained in 5-gallon stainless steel aquariums at 20–21° C. All fish were fed mussels, on which they thrived. Low oxygen concentrations were obtained in the experimental aquariums by boiling sea water or bubbling nitrogen through it, which is perhaps preferable in that the organic constituents remain relatively unaltered. Dissolved oxygen was measured with a commercial galvanic type oxygen analyzer (*cf.* Duxbury, 1963).

Aerial respiration

We observed the mudsucker's general behavior leading to gulping air at the water surface among a group of 13 fish. We tallied their progressive reactions to each of 7 different oxygen concentrations, which constituted a stepwise decrease from 2.8 to 1.1 mg./l. In another experiment, fish were immediately placed in deoxygenated water (0.5 mg./l.) to determine the necessary period of adjustment before the initial gulp.

To measure the consumption of aerial oxygen, 4 fish, 117–165 mm. in total length, were put into critically deoxygenated water (less than 1.0 mg./l.). When oxygen concentrations were low enough to apparently preclude aquatic breathing (*i.e.*, opercular movements were undetectable), the fish would periodically rise for atmospheric air. Then by immersing them tail first with their mouths held open, all air was forced from their buccopharynx and they were confined, one at a time, within a partly submerged, water-filled, 6-inch glass funnel, which was inverted in an aquarium. Gulp and expelled air was retained in the apex of this funnel whose exposed, cut-down spout was capped by a rubber diaphragm (Fig. 1). After the water level in the aquarium was adjusted to preclude any volumetric change in it, 3 or 6 ml. of air were injected by hypodermic syringe through the diaphragm into the exposed cap of the funnel, thereby displacing an equal volume

of water and registering volumetrically at the apex calibrated in ml. This volume was kept minimal to insure the most accurate possible determination of its percentage oxygen change. For the 117-mm. fish (25.5-g.) the volume injected was 3 ml. For the 140- and 165-mm. fish (30- and 36-g.), however, it was necessarily increased to 6 ml., so that the fish could project their mouths above the surface to gulp in the confined space. Gulping would always occur quickly and the volume of air taken was measured by noting the water displacement in the apex; this volume

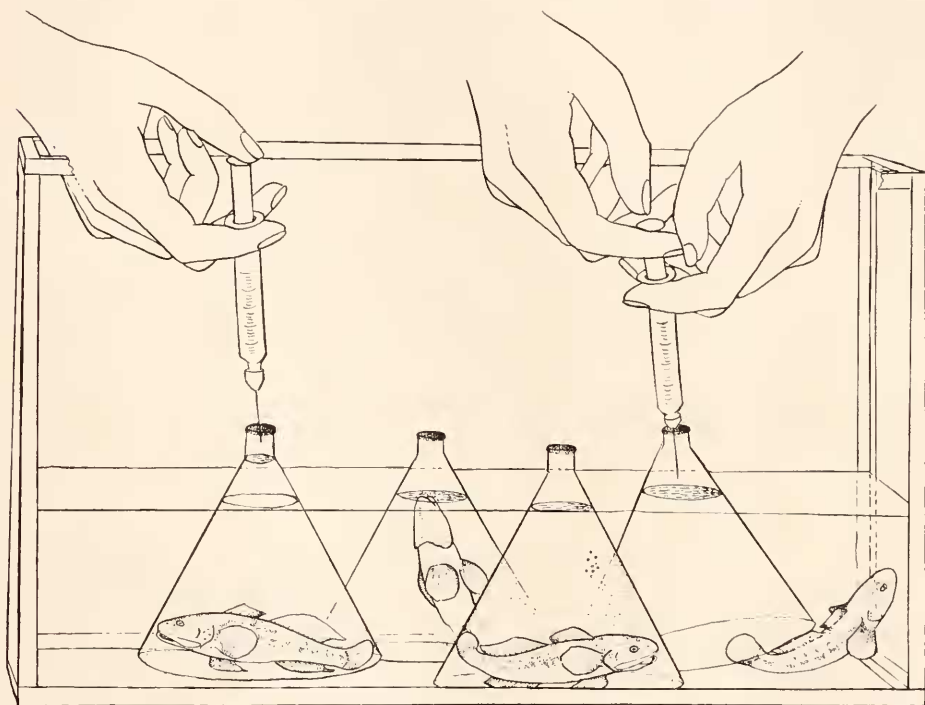


FIGURE 1. Determination of aerial oxygen consumption in *Gillichthys mirabilis*. From left to right: an inoculum of air is provided a gulping fish restrained under a filled funnel in deoxygenated seawater; the fish soon gulps this air in the funnel apex; it returns to the bottom, retaining the bubble but expelling excess air; after a brief sojourn it releases the bubble and is allowed to escape, so that the altered volume of air in the funnel can be analyzed for per cent oxygen and carbon dioxide.

was retained intact by carefully lifting the funnel over and away from the fish. Only those experimental runs were accepted in which the fish released all of the air held. After the fish had expelled all the retained gas under the funnel, the percentages of oxygen and carbon dioxide in the resulting new volume of gas at the apex were measured with a Scholander microgasometric analyzer (Scholander *et al.*, 1955), after first removing a 2-ml. sample with a 5-ml. syringe.

Before each successive observation, all air was again forced from the fish's buccopharynx. During the four runs, each with a different fish and including 7-12 complete observations, we noted the elapsed time between gulping and expelling,

i.e., the time that the fish remained submerged with the bubble. Linear regressions were then calculated of the percentage of oxygen remaining in the inoculum of air and of the volume of oxygen extracted from the bubble on time submerged, and of the volume of carbon dioxide expired on that of oxygen consumed.

Volumes were calculated using the reasonable assumption that the original inoculum contained 21% oxygen and 0.03% carbon dioxide; gasometric analyses of air bubbled into the funnel confirmed the negligible alteration of these percentage volumes through the water. The volume of these gases respired between gulping and expelling, therefore, could be approximated from the formulas:

$$V = \frac{I(0.21 + P') - P}{1.0 - P} \quad \text{and} \quad V' = \frac{I(0.03 - P') + PV}{P - 1.0}$$

(noting that calculation of V' depends on first calculating V), where V equals the volume of oxygen respired, V' the volume of carbon dioxide expired, I the volume of inoculum (3 or 6 ml.), P the percentage of oxygen in the funnel after expiration, and P' the percentage of carbon dioxide after expiration. Because it was usually impossible to measure small volumetric changes on the roughly calibrated funnel apex, the calculated volumes respired were generally unsubstantiated by direct observation.

To further investigate the onset and control of gulping, the two larger fish were placed in a 3-gallon all-glass aquarium fitted with a gas-tight lucite lid drilled with a gas inlet, outlet, and a stoppered center aperture for monitoring temperature, salinity, and gas concentration in the water. After the water was deoxygenated (less than 0.5 mg./l.) by bubbling with nitrogen, the fish were placed one at a time under an atmosphere of pure nitrogen maintained in a 2-inch space above the water level. After recording their reactions and allowing them to readjust to normal conditions, they were placed under a 1:1 mixture of nitrogen and carbon dioxide. All gases were piped into the experimental aquarium from adjacent pressure tanks fitted with demand valves. Later, the aquarium cover was removed and the water containing the fish was aerated with the same gases; first nitrogen, then the mixture. We observed the frequency and pattern of surface gulping by each fish under the four conditions.

Following the preceding experiments, in which the air in the funnel was renewed after each gulping sequence, another in which the air was not renewed was undertaken to determine the efficiency of aerial respiration during a continual increase in respiratory carbon dioxide concentration. Oxygen uptake from, and carbon dioxide excretion into the bubble were calculated and the behavior of the subject was noted during the course of the experiment (about 30 minutes) when the fish had access to only the original inoculum of air. Also, the size of gulp was compared with the resulting depletion of oxygen in the funnel apex after the bubble was subsequently released.

To investigate possible differences in rates of oxygen consumption between individuals that are emergent but moist (reported by Barlow, 1961a, for fish breathing pure oxygen) and others that are submergent but gulping air, oxygen uptake was also measured among four fish (all about 90 mm. in total length, weighing 7.45–10.95 g.) in a Mark brand Scholander microvolumetric respirometer. The regular tubular chambers of the apparatus were replaced by 250-ml. reagent bottles with

ground glass fittings to hold the half-grown fish, so that they would have ample room in which to move about. A potassium hydroxide boat in each chamber removed excess carbon dioxide during the experimental runs. Following Barlow's procedure, the fish were not fed for several hours before the experiment. Each fish was blotted gently and weighed before each run. Two daylight experiments, each comprising 40 observations (10 observations each among four fish), were carried out at $20 \pm .03^\circ \text{C}$. in a constant-temperature bath: one in moist chambers containing about 5 ml. of unfiltered sea water and atmospheric air, where the fish had to breathe aurally without gulping; the other in chambers half-filled with deoxygenated sea water, where they gulped air at the surface. The fish continued gulping throughout the second experiment, even though the "deoxygenated" water became progressively aerated (from 0.8 to 2.15 mg./l.) as a result of the fish's motion in the chamber. All fish were placed in the respirometer chambers 48 hours before the first measurement, which was subsequently discarded because the fish were hyperactive when first approached. The fish soon adjusted to the experimental regime, however, and subsequent measurements showed no significant "within fish" differences in oxygen consumption among the 15-minute trial periods. These relatively short trials probably caused the fish to be in a continuous state of active metabolism during the entire experiment, which, according to Barlow, implies that their oxygen consumption should be about twice that during a resting state of standard metabolism. After each trial the excursion of the piston indicator of chamber pressure was read, the chamber was flushed with air and reactivated, the fish was allowed a short period of readjustment, and the next trial was initiated. The four fish were observed concurrently during each experiment. Possible differences among trials, animals, and treatments (experiments) were tested in an analysis of variance and the recorded oxygen consumption was compared with rates (cc./kg. body weight/hr.) presented elsewhere.

Structure of the buccopharyngeal organ

Gross dissections were made of the buccopharyngeal region only insofar as its structure is directly concerned with aerial respiration. The extent and development of vascularization in the buccopharyngeal epithelium of fish from deoxygenated water was compared with that of others from aerated water. Also, the buccopharynx was dissected and carefully examined after the fish had been injected intracardially with black India ink. (Half-grown fish were not injected because their integument was relatively transparent.)

Superficial vascularization in *G. mirabilis* was then compared with that of similarly injected specimens of the killifish *Fundulus parvipinnis*, which is an obligatory aquatic breather and locally is sympatric with *G. mirabilis*. The general extent of buccopharyngeal vascularization in the two species was compared after individuals had been placed in deoxygenated water. For a histological comparison, small squares of epithelium were excised from the posterior part of the capillary bed in the buccal roof of each of 6 fish (three of each species) that had been living in poorly oxygenated water and four fish (two of each species) from aerated water. These 10 epithelial squares were fixed in Bouin's solution and two from each group (8 total) were chosen at random to be embedded in paraffin, stained in Mallory's triple stain, and sectioned at 10μ .

Composition of gas in the swimbladder.

We investigated the possible function of the swimbladder as an auxiliary respiratory organ because: (1) the fish must have an oxygen source during its adjustment period before gulping, and (2) its red gland (gas-secreting and -concentrating organ in the swimbladder) is enlarged relative to that of most other littoral fishes.

First, the swimbladder gas composition of fish in aerated water was compared with that of others in deoxygenated water. Four pairs of adult fish were removed from an aerated aquarium and placed in continuously aerated bowls of sea water. After 15 hours a 0.5-ml. sample of gas was drawn from the swimbladder of one fish of each pair by coelomic puncture with a 5-ml. hypodermic syringe while the fish was held firmly underwater. This was analyzed for the percentages of oxygen and carbon dioxide in the microgasometric analyzer. Meanwhile, the second fish were placed in deoxygenated water for periods ranging from 0.5–15 hours, after which they were removed for swimbladder gas analyses.

A comparison of swimbladder gas among fish living in differing environments was made by analyzing gas samples from fish in an aerated aquarium, a deoxygenated aquarium, a large circular outdoor concrete tank with stratified layers of different oxygen concentrations, the campus lagoon, and an aquarium under an atmosphere of pure nitrogen. Also, gas content was measured among groups of 5 fish each at 6, 12, 24, and 48 hours after their swimbladders had been emptied by coelomic puncture.

The approximate volume of the swimbladder was measured as the maximum amount of gas removable by a syringe from fish in shallow water.

RESULTS

Behavioral responses to low oxygen concentrations

Our general observations indicate that adult mudsuckers always respond to critically low oxygen concentrations (less than 1.6 mg./l.) by breathing aerially; they cease all opercular movements, gulp mouthfuls of air at the surface, then usually descend for a sojourn on the bottom, and finally release the spent air through the mouth before the next ascent. (Occasionally, in water containing very little oxygen, they remain vertically suspended with snouts contacting the surface.) When denied access to the surface by, for example, a plastic screen, they suffocate when the oxygen concentration is lowered much below the critical level. Young and halfgrown (to 90 mm. total length), however, are facultative gulpers and often continue opercular movements and remain at the surface while apparently breathing aerially. Unlike a typical non-air-breathing fish which usually reacts violently when first removed from water, the mudsucker remains motionless and gulps air at a measured pace. The capacity of its buccopharyngeal cavity can be increased by a strong depression of the tongue and hyoid bones, resulting in a gular expansion of more than 50% of the total volume of the cavity. Like other air-breathing gobies, the fish tends to hold the air anteriorly in this cavity and props its head slightly higher than its tail.

Specifically, when dissolved oxygen fell below 2.0 mg./l. in the experimental aquarium, the cessation of the fish's opercular movements usually preceded the

onset of surface gulping by several minutes. Moreover, the rate of opercular movement did not appreciably change as the oxygen concentration was gradually lowered to critical levels: at 5.6 mg./l., opercular beats among 5 aquatic breathers averaged 58.2/min. (range, 48-78); at 4.0 mg./l., 48.5 (40-64) among the same fish; at 2.8 mg./l., 59.5 (38-84); at 2.2 mg./l., 57.5 (40-80) among these fish, one of which was now abortively gulping; at 2.0 mg./l., 53.4 (36-76) among the three fish that were still breathing aquatically; and at 1.8 mg./l., 60.7 (36-76) among these three, two of which were now gulping sporadically. It is emphasized that even when adults began gulping in oxygen concentrations greater than about

resp. act. O ₂ , mg./l.	opercular movements?				
	y e s			yes, no	no
	resting on bottom	contemplat- ing surface	gulping, expelling	gulping, expel- ling or retaining	gulping, retaining
2.8	12	1			
2.2	9	1	3		
2.0	6		3	3	1
1.8	5	1		6	1
1.6	1	2			10
1.4		2			11
1.1					13

FIGURE 2. Progressive reactions of 13 mudsuckers to successively lowered dissolved oxygen concentrations. The hatched areas represent proportions of fish exhibiting designated reaction; the numbers in the blocks are the actual counts.

1.6 mg./l., opercular movements often continued at the normal rate. Preliminary to the functional gulping, about half of the fish performed abortive intention movements, obviously related to gulping behavior (Fig. 2). Before oxygen had fallen to the critical level, they breathed normally and often rested quietly on the bottom, supported and anchored by their fused pelvic fins, which function as suction cups. As the oxygen was decreased below 3.0 mg./l., however, they would either begin to glance upward, then swim toward but not to the surface, or they would simply approach the surface without the preliminary glancing. Then, as the concentration was further decreased, some fish would break the surface and gulp air, which, however, was almost immediately expelled through the mouth. Finally, at the critical concentration of about 1.6 mg./l., they would assume the normal aerial response of gulping mouthfuls of air, which were held for an average of 3.5 minutes. Interestingly, the other fish either forewent some of the preliminaries or simply assumed the functional gulping response immediately.

The adult fish that were quickly transferred from aerated to deoxygenated water hesitated 2–58 minutes (usually 15–20) before gulping, during which time opercular movements ceased. Half-grown fish also hesitated for comparable periods, but their opercular movements continued longer. While gulping, several fish floated almost vertically at the surface with their snouts above water; this behavior was repeated on several occasions.

Consumption of aerial oxygen

Expectedly, aerial oxygen was consumed in almost direct proportion to the duration of bubble retention, which varied from 0.83–10 minutes during the four experimental runs and was apparently dependent on the volume of air carried to

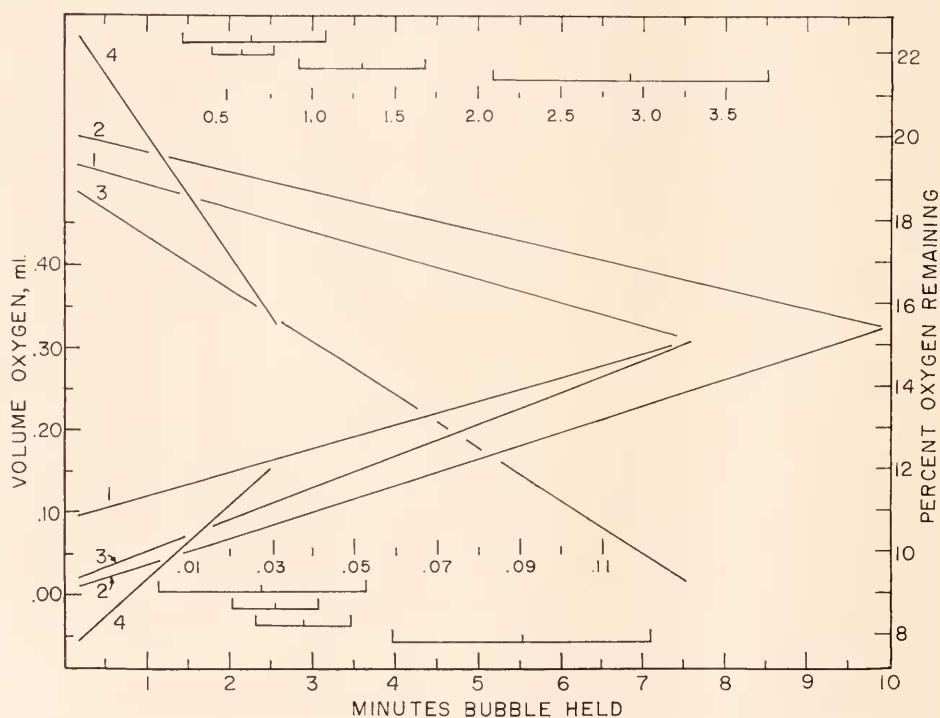


FIGURE 3. Least squares regressions of (right ordinate) per cent oxygen remaining in a volume of air in the funnel apex after the restrained mudsucker has released its bubble (upper four regression lines) and (left ordinate) the calculated volumes consumed during its bottom sojourn (lower four lines), on (abscissa) the time that each bubble was held. Numbers at the left extremes of the lines designate: 1, 165-mm. fish (7 observations while gulping in 6 ml. of air); 2, 140-mm. fish (8, in 6 ml.); 3, 117-mm. fish (12, in 3 ml.); 4, a second 117-mm. fish (9, in 3 ml.). The series of four measured horizontal lines at the top and bottom of the graph denote the regression coefficient "b" (centered short vertical line) and 95% confidence limits (horizontal extremes) for fish 1 (uppermost horizontal line in each series) through 4 (lowermost line). Non-overlap of any pairs of horizontal lines, therefore, indicates significant differences in b's (slopes of the lines). All regressions are significant at: 1, $P = 0.025$; 2–4, $P < 0.001$ levels.

depth by the fish. This volume, in turn, was erratically determined by the fish's success in securing an optimal volume in the initial gulp; *i.e.*, if too much air is retained (1.0 ml. or more), the fish is positively buoyant and cannot maintain its resting position on the bottom, and, conversely, if only a small amount of air is retained (less than 0.5 ml.), the fish lacks sufficient oxygen to allow it a reasonable sojourn on the bottom. Therefore, if too much was initially gulped, the positively buoyant fish would expel excess air during its descent or on first reaching the bottom. Occasionally the fish would overcompensate by expelling too much air and thereby shorten its bottom sojourn. When the funnel was removed, the duration of sojourn varied similarly: 0.5–10 minutes (average 3.8, usual range 2–5, 35 observations) compared with the above-mentioned durations under the funnel, 0.83–10 minutes (average 3.5, usual range 2.5–5.0, 36 observations). When a fish expelled part of a fresh bubble during hydrostatic adjustment, it was released from the mouth, operculum, or both. A respired bubble that was retained during the sojourn, however, was always released from the mouth.

For each trial, the regression (Fig. 3) of the percentage of oxygen in the funnel after expiration (Y) on duration of bubble retention (X) is highly significant ($P = 0.01$ – 0.001). Furthermore, the Y -intercepts ($a = 18.91$ – 22.89), which represent the theoretical percentage concentration of oxygen in the original inoculum of air, are reasonable values, even though that for one agitated 117-mm. specimen was significantly greater than the rest. To be sure, the obvious differences among regression coefficients ($-b = 1.297$ and 2.918 for the two smaller fish, 0.578 and 0.632 for the two larger) might partly be due to the fact that the smaller fish had less air to breathe than the larger; *i.e.*, all else being equal, the rate of percentage change in the small volume is necessarily greater (Fig. 3, horizontal lines at top). Therefore, the percentage decrease in aerial oxygen was converted to the presumed actual volumes consumed. Although less variable than those based on per cent change, the regressions of volumes on time held also indicate differences among regression coefficients (Fig. 3, horizontal lines at bottom), which of course are now positive ($+b = 0.038$ and 0.091 for the smaller fish, 0.026 and 0.031 for the larger). Both of the larger and one smaller fish consumed oxygen at about the same rate (as measured by the regression coefficients) throughout the experiment. The obvious hyperpnea in the second smaller fish, however, is substantiated by its behavior; it appeared agitated and had sojourns averaging only 1.73 minutes, compared with 3.77 minutes for the first and 5.11–5.27 for the two larger fish. Possible curvilinearity in some regressions is implied by the variation of Y -intercepts, which, of course, should theoretically equal zero.

Percentages and volumes of carbon dioxide increased in almost direct proportion with oxygen consumption (Fig. 4). The regression coefficient of carbon dioxide (Y) on oxygen (X), which essentially equals the respiratory quotient for the fish, however, was less (0.279) than would be expected if all gaseous exchange occurred in the pharyngobranchial epithelium. However, most of the carbon dioxide may have been excreted through the gill membrane (*cf.* Carter, 1957), some through the skin (*cf.* Krogh, 1904) and a significant amount dissolved in sea water while the bubble was being "mouthed" and expelled. (The theoretical average RQ, 0.83, for the metabolism of proteins, fats, and carbohydrates (*cf.* Baldwin, 1949) approximates the average of values, 0.76, obtained by Schöttle (1931) for the

mudskipper *Periophthalmus* breathing in a closed system where exchange through all respiratory membranes was measured.)

In deoxygenated water under an atmosphere of pure nitrogen, the larger fish, after waiting 8–15 minutes, gulped and held the nitrogen bubble for an average of 5.40 minutes during 5 trials. But under a 1:1 mixture of nitrogen and carbon dioxide, they held for an average of only 34 seconds, then remained at the surface with their mouths above water. In an uncovered aquarium of oxygenated water

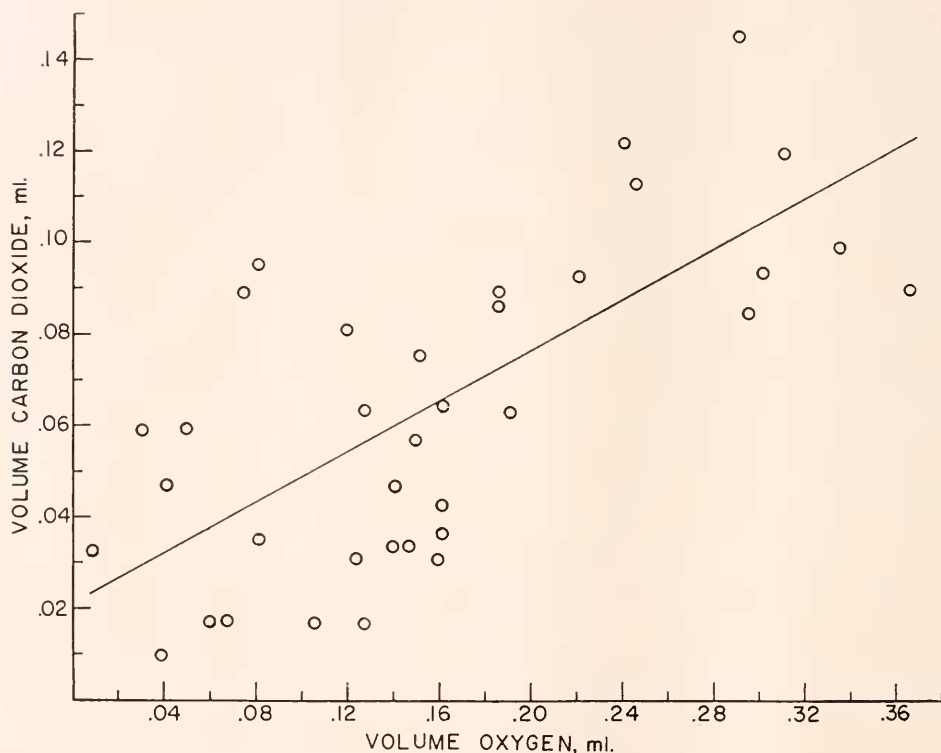


FIGURE 4. Regression of calculated volumes of carbon dioxide expired on those of aerial oxygen consumed per gulp for the four mudsuckers gulping under inverted funnels. The regression coefficient is 0.279 and the regression is significant at $P < 0.001$.

aerated with nitrogen they were unaffected while the oxygen concentration was sufficient for aquatic respiration, but began gulping when it became critically low. When the aquarium was aerated with carbon dioxide, however, the fish gulped within 10 minutes and showed the dilation of buccopharyngeal vascularization that is always preparative for gulping.

While gulping the same air for 30 minutes under the funnel, the larger fish increased the volume per gulp from 0.5–1.0 ml. to 1.0–2.0 ml. Near the end of this experiment, however, the fish gulped erratically, expelled bubbles quickly, and did not regularly either increase or decrease the volumes gulped. During this period,

the oxygen in the funnel air decreased to 11% of the remaining volume and carbon dioxide increased to 3%.

An analysis of variance revealed no significant differences in respirometrically determined oxygen consumption between four half-grown fish breathing aerially in moist chambers and the same individuals gulping in chambers half-filled with sea water (Table I). Also, during the two experiments the lack of significant "within-fish" differences indicated no detectable change in rate of oxygen consumption with time. The significant differences among the four fish ($P = 0.01$) that were revealed in a two-way analysis eliminating replications (within-fish trials) were, perhaps, due to differences in excitability; whereas the two 8-gram fish consumed an average

TABLE I

Variation in oxygen consumption in Gillichthys mirabilis, either emergent and moist or submergent and gulping, as expressed by an analysis of variance: upper, 3-way with the third-order interaction used as the "error" term and lower, 2-way with replications (trials) pooled and eliminated (NS, not significant at the $P = 0.05$ level)*

Source of variability		Sum of squares	Degrees of freedom	Mean square	"f"	P
A. Among individual fish		10214.1	3	3404.7	4.2	0.025
B. Among replications (trials)		3488.2	9	387.6	<1	NS
C. Between treatments (emergent or submergent)		2063.8	1	2063.8	2.5	NS
Interactions	A × B	17751.7	27	657.5	<1	NS
	A × C	1635.0	3	545.0	<1	NS
	B × C	10617.6	9	1179.7	1.4	NS
	A × B × C	21949.8	27	813.0		
"Error"						
A. Individuals		10214.1	3	3404.7	4.55	0.01
C. Treatments		2063.8	1	2063.8	2.76	NS
Interaction: A × C		1635.0	3	545.0	<1	NS
Error		53807.2	72	747.3		

* The small number of samples (one/cell) disallowed a 3-way analysis with true error term.

of 100 (range 80–136) and 91 (50–123) cc./kg./hr., respectively, the two 10-gram fish surprisingly consumed oxygen at a significantly faster rate: 113 (64–174) and 112 (63–172) cc./kg./hr. All fish averaged 104 (50–174) cc./kg./hr. for all trials. Our values approximated those obtained by Schöttle (1931), 94–210 (extrapolated to a body weight of 8–10 g. at 20° C.), for *Periophthalmus* and those of Wells (1935), 96–116, for aquatic breathing mudsuckers acclimated to 10.5–13.0° C. However, they were less than twice the average, 65 (interpolated at 20° C.), that Barlow (1961a) determined for emergent but moist fish breathing pure oxygen and Wells' range of values, 62–68, for aquatic breathers acclimated to 32.8–34.5° C. Whereas we as well as Schöttle accepted measurements taken during the first 2 hours of trials, Barlow rejected those of the first 12 hours, during which the rate of oxygen consumption generally decreases by half from an active to a standard rate. In evaluating the respiratory potential of the swimbladder, therefore, we assumed

that a half-grown mudsucker consumes oxygen at a standard rate of about 65 cc./kg./hr. and a large adult at about 45 cc./kg./hr.

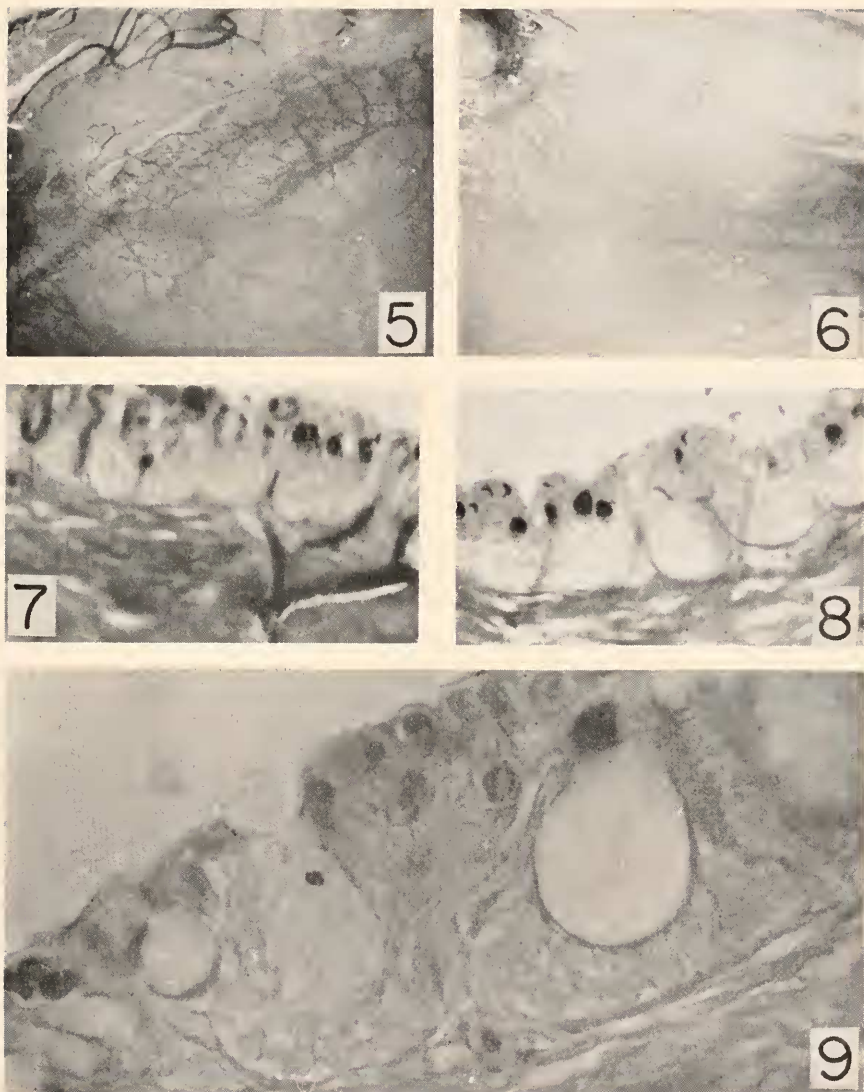
Structure of the buccopharyngeal organ

The large and loosely hinged jaws of *G. mirabilis* form a relatively huge gape, whose full lateral expansion measures almost half the body length and serves in a behavioral "combat pose" during courtship (Weisel, 1947). Its wide, heavily vascularized frenum could extend the aerial respiratory surface (Weisel), but our observations indicate that this membrane is not exposed to the air bubble. The marked depressibility of the floor of the mouth allows considerable expansion of the buccopharyngeal cavity. Unlike *Periophthalmus*, whose pharyngeal respiratory surface is extended by a fold of skin attaching the first gill arch (Schöttle, 1931), *Gillichthys* has four complete and normal gill arches. Like *Periophthalmus* and other air-breathing gobies, however, its gills are relatively short, comprise only about two-thirds the relative surface area of those of many obligatory aquatic species, and tend to clump during emergence.

The capillary beds that permeate the buccopharyngeal organ are simply extensions of vessels whose homologues in obligatory aquatic breathers are not so proliferous. The epithelium is profusely vascularized, particularly a triangular area in the buccal roof, which contacts the top of the engulfed air bubble (Fig. 5). When injected with India ink or engorged with blood, this network is conspicuous compared with homologous vascularization in an obligatory aquatic breather such as *Fundulus* (Fig. 6). Even when uninjected, furthermore, the superficial vessels, which are enveloped in silvery-black pigment, are clearly visible, especially so in smaller specimens.

Afferent vessels supplying the respiratory epithelium include branches of the carotid, hyomandibular, hypobranchial, and segmental arteries. From the ventral aorta, blood courses the first efferent branchial artery to the hyomandibular artery, which, through its mandibular and lingual branches, supplies the rich capillary beds in the tongue and ventrolateral buccal epithelium. Also, the hyomandibular artery, together with hypobranchial branches of the second and third efferent branchial arteries, supply smaller networks in the pharyngeal epithelium. The vasculose superior buccal epithelium is fed by orbital, nasal, and smaller branches of the carotid arteries, which originate from the dorsal aorta at the first efferent branchial artery. Although in mudskippers the orbital and nasal arteries originate together from the orbitonasal branch of the internal carotid (Schöttle, 1931), in *G. mirabilis* no common source supplies these vessels, which originate separately from the internal and external carotids, respectively.

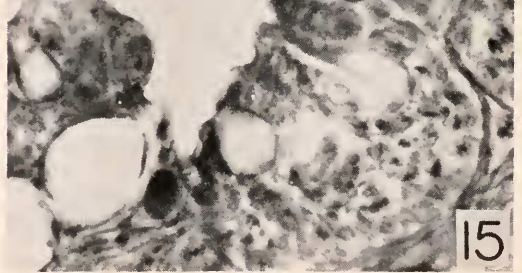
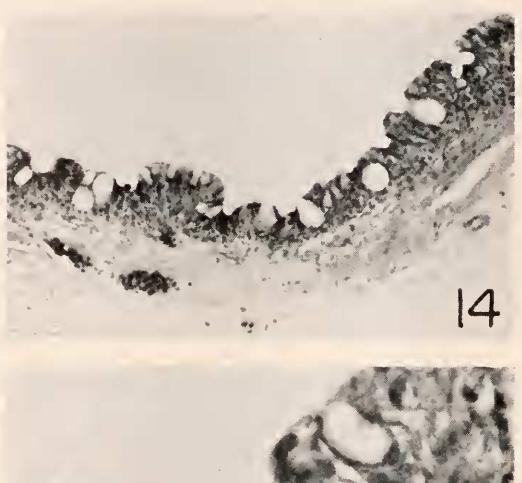
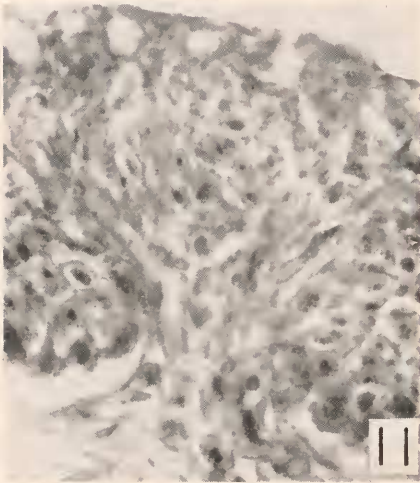
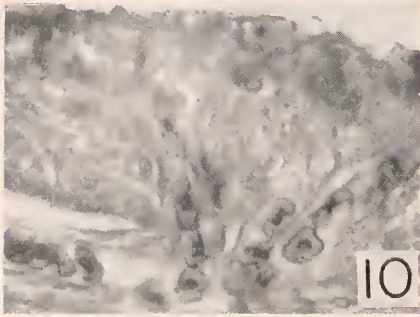
Efferent vessels draining the respiratory epithelium include the internal and external jugular veins, the orbital sinus, the opercular vein, and their tributaries. Lingual and other branches to the internal jugular vein drain the capillary beds in the tongue, inferior buccal epithelium, and hypobranchial region. The orbital sinus and external jugular trunk, which admit orbitonasal and nasomaxillary branches, respectively, receive blood from the superior buccal epithelium, while laterally, two large branches to the external jugular drain the periphery of this region. In the pharynx the large opercular vein and its tributaries drain into the anterior cardinal sinus, which also drains the orbital and external jugular sinuses.



FIGURES 5, 6. Medio-lateral surfaces of the buccal roof from: 5, *Gillichthys mirabilis* and 6, *Fundulus parvipinnis* after both fishes were taken from deoxygenated water and injected intracardially with India ink. $\times 60$.

FIGURES 7, 8. Cross-sections, from the postero-medial buccal roof, of respiratory epithelium (lighter upper tissue) and dermal corium (darker lower tissue), showing capillaries (dark vertical lines) extending from the corium to the epithelial surface. In: 7, from a mudsucker beginning to gulp in deoxygenated water, the capillaries are engorged with blood; 8, from a fish returned to aerated water, they are collapsed. $\times 430$.

FIGURE 9. Cross-section of buccal-roof epithelium from a gulping mudsucker taken from deoxygenated water, showing enlarged vacuoles, one of which (to left of middle) is secreting mucus, interspersed cuboidal cells, and erythrocytes in engorged capillaries (e.g., dark-staining cells near surface). $\times 1675$.



FIGURES 10-15. Cross-sections of buccal-roof epithelium from a: 10, mudsucker taken from aerated water but with partly filled epithelial capillaries ($\times 1150$); 11, fish from aerated water with collapsed capillaries and contracted vacuoles ($\times 1430$); 12, killifish, *Fundulus parvipinnis*, from deoxygenated water, showing one taste bud (dark-tipped striated oval at right), but no epithelial capillaries and with small vacuoles under a smooth epithelial surface ($\times 1430$); 13, mudsucker from aerated water with contracted vacuoles and a smooth epithelial surface ($\times 270$); 14 ($\times 270$), 15 ($\times 1150$), fish from deoxygenated water with expanded vacuoles and corrugated epithelium. In all figures the strongly nucleated epithelium overlies the fibrous corium. The dermal and epithelial capillaries (tubular structures) contain erythrocytes (oval cells with darkly-staining nuclei). Several white vacuoles open to the epithelial surface.

Schöttle (1931) described three progressively more effective respiratory modifications of the buccopharyngeal epithelium in gobies: (1) the capillaries are relatively sparse and completely subepithelial in mostly aquatic *Gobius caninus* and *Periophthalmus koelreuteri*, (2) they are more numerous and extend from the subtending corium to the epithelium in amphibious *Boleophthalmus boddarti*, and (3) they are completely epithelial in semi-terrestrial *P. schlosseri*. In the more competent air-breathers, extensive epithelial corrugation enhances this capillary profusion by greatly expanding the respiratory surface. The superior buccal epithelium of *G. mirabilis* most closely resembles the structurally intermediate type of *Boleophthalmus*, in that many capillaries extend from the dermal corium to the surface of the glandular epithelium, where they course among the vacuolated cells and often contact the surface membrane (Figs. 7, 8, 10). In contrast with the mudskipper, however, vascularization appears denser in the buccal (rather than pharyngeal) epithelium. Almost all capillaries in fish from deoxygenated water are expanded (Fig. 7), whereas those of fish in aerated water appear mostly collapsed (Fig. 8).

A comparison of histological sections from the buccal roof of *G. mirabilis* with others from obligatory aquatic *Fundulus parvipinnis* revealed manifold differences, especially among specimens from deoxygenated water. Within 10 intervals of 6 mm. each, *Gillichthys* averaged 65 epithelial capillaries, many of which approached the surface, whereas *Fundulus* had few or none contacting the epithelium and those that did were often associated with taste buds. The epithelium of both species is composed of cuboidal cells and interspersed mucus-secreting goblet cells (Figs. 9–11). Specimens of both from aerated water have smooth epitheliums containing relatively small vacuoles (Figs. 11–13). But among others from deoxygenated water, only specimens of *Gillichthys* showed a noticeable epithelial transformation: vacuoles were enlarged to 5–10 times normal size (Figs. 9, 14, 15), and the numerous epithelial capillaries were engorged with blood. In this condition it resembled the thickened corrugated epithelium of *P. schlosseri*, which is similarly permeated by branched, alveolar-like cavities and engorged capillaries (*cf.* Schöttle, 1931; Harms, 1935). When fully expanded, the vacuoles open to the epithelial surface and exude over it a copious mucus (Fig. 9), which may provide the fluid medium of respiratory exchange.

When *Gillichthys* enters either deoxygenated water, water aerated with carbon dioxide, or air, its buccopharyngeal capillaries, as they become engorged with blood, dilate and redden. Within 3–5 minutes superficial capillaries impart a rosy hue to the tongue and buccal roof; conversion of the larger vessels is completed 10–20 minutes later. Although this response is relatively rapid, its reversal in aerated water requires several hours or occasionally even days, during which the red-mouthed, transient fish are easily distinguishable from the white-mouthed residents and often gulp sporadically. No such vascular transformation accommodates the buccopharyngeal epithelium of *Fundulus*.

Composition of gas in the swimbladder

In the mudsucker's physoclistic swimbladder, the glandular part (red gland), which comprises a round gas gland and long unipolar rete mirabile (series of

TABLE II

Proportions of swimbladder oxygen in gulping and aquatically breathing mudsuckers among various habitats (average precedes the range, in parentheses, and number of individuals sampled)

Habitat	Per cent oxygen in swimbladders of fish:	
	Gulping in low oxygen, respiratory capillaries engorged with blood	Respiring aquatically in normal oxygen, capillaries not engorged
Aquarium, 1 month	17.9(15.5-20.8)6	26.2(18.0-34.4)10
Large concrete tank, 1 month	22.5(22.0-23.5)3	32.7(30.5-34.0)3
Campus lagoon, immediately after capture	16.0(14.0-26.0)6	25.7(24.3-28.4)6
Grand average	18.1	27.1

minute, juxtaposed capillaries where gases are concentrated) is well developed and, therefore, should have a relatively good capacity for oxygen secretion.

After the two groups of four fish each had been transferred from an aerated aquarium into either deoxygenated or continuously aerated sea water, their swimbladder gas differed markedly in oxygen content. Swimbladders of fish in aerated water contained 40.5-43.0% 15 hours after transfer, whereas those of fish in deoxygenated water contained significantly less: 19.0-23.5% in two fish after 27-29 minutes, 27.2% in one after 80 minutes, and 21.0% in the last after 15 hours. The group in deoxygenated water remained quietly submerged without noticeable opercular movement for 8-33 (average 15.3) minutes before gulping. In a third

TABLE III

Proportions of newly secreted oxygen and carbon dioxide in refilled mudsucker swimbladders at selected intervals after the forced removal of the original gas

	Time after removal, hours			
	6 (Residual gas?)	12 (Refilled normal volume)	24	48
Oxygen, %	33.6	85.4	85.5	41.5
	31.0	85.4	84.3	39.3
	31.9	81.4	73.3	56.5
	39.5	84.2	81.4	73.0
	23.0	78.3	84.4	57.5
Average	31.8	82.9	81.8	55.0
Carbon dioxide, %	2.6	13.5	12.0	4.2
	4.5	9.3	10.0	6.0
	3.7	13.0	3.2	4.0
	3.5	6.2	2.6	5.0
	5.0	9.5	3.2	5.5
Average	3.9	10.3	6.2	4.9

group placed in deoxygenated water, swimbladder oxygen fell from an average of 28.5% to 14.0% in 17 minutes, their average time to gulping. In deoxygenated water, obligatory aquatic-breathing killifish, *Fundulus parvipinnis*, and topmelt, *Atherinops affinis*, died in less than 15 minutes, during which no proportional change in their swimbladder gas content was detected.

Swimbladder gas in 19 fish gulping in poorly oxygenated water among different environments averaged only 18.1% oxygen, compared with 27.1% in fish (excluding those with recently filled swimbladders) from normally oxygenated water (more than 5.0 mg./l.) in similar environments (Table II). The percentage volume of carbon dioxide averaged 2.7% in the fish from poorly oxygenated water, 2.3% in those from normal water. After 20 minutes in deoxygenated water under a nitrogenous atmosphere, one fish, after remaining submerged for 15 minutes before gulping, had 6.0% oxygen in its swimbladder; another, which gulped after 45 minutes, but 1.5%.

Six hours after they had been deflated, swimbladders in 5 fish contained only about 10–14% of the filled volume of gas. Because the gas averaged only 31.8% oxygen and 3.9% carbon dioxide, furthermore, it was assumed that most of it was residue from incompletely deflated organs. In 12 hours the swimbladders had completely refilled with gas averaging 82.9% oxygen and 10.3% carbon dioxide (Table III). Subsequently, however, the percentages decreased proportionately to 48 hours, when oxygen averaged 55.0% and carbon dioxide 4.9%, as compared with "normal" averages of 26.2% and 2.3%, respectively (Tables II, III).

The volumes of 5 swimbladders measured by attempted complete gas removal into a calibrated syringe constituted 3.0–3.6% of the body volumes.

DISCUSSION

Carter (1957) proposed three criteria that ideally should be satisfied in determining the respiratory capacity of suspected internal air-breathing organs in fishes: (1) the organ's epithelium should be richly supplied with blood which is better oxygenated after leaving the organ, (2) its cavity should be regularly ventilated, and (3) its expelled air should contain proportionately less oxygen and more carbon dioxide than atmospheric air. Also, he acknowledged the importance of biological and behavioral evidence, such as an established pattern of presumed air-breathing movements and the ability to survive in deoxygenated water if allowed access to surface air (or inability if denied such access). Of these, we have investigated all but the criterion of blood gas concentrations, which will be the subject of later studies.

Although all fishes tend to gulp or gasp at the surface in deoxygenated water (*e.g.*, Fry, 1957; Jones, 1964), only the species specifically adapted for aerial respiration can continuously survive by such actions. When dissolved oxygen falls below 2.0 mg./l., for example, an adult mudsucker usually gulps air at the water surface (often after a preliminary sequence of abortive "intention movements"), holding the bubble in its large buccopharyngeal cavity and retiring to the bottom for varying periods until the bubble is expelled. If denied surface access it suffocates if the surrounding concentration of dissolved oxygen is less than 1.6 mg./l. Among other fishes, similar responses are achieved by the anabantoids, such as the paradisefish *Macropodus opercularis* of the swamps and rice paddies of southeastern

Asia, whose air-breathing organ comprises complex, heavily vascularized lamellae set in a spacious suprabranchial cavity (*e.g.*, Bader, 1937; Forselius, 1957).

Thompson (1925) concluded that water containing less than 2.0 p.p.m. (mg./l.) of oxygen will kill many kinds of obligatory aquatic breathers and, according to Ellis (1937), "ample and favorable" natural waters should contain more than 5.0 mg./l. of oxygen to be generally suitable for most fishes. Jones pointed out that previous work has shown the tolerance of low oxygen tensions to be a characteristic that is best developed in relatively sluggish fishes and is strongly influenced by the ambient temperature, although even for these types optimal concentrations are much greater than the lowest tensions survived. The low tensions that induce aerial respiration in *G. mirabilis* approximate critical tensions that cause discomfort, suspension of normal activities, and often death in many non-air-breathers. Also, in contrast with most fishes, which are oxykinetic (made restless by decreasing oxygen) and are thereby stimulated to randomly seek better oxygenated water (Hoglund, 1961; Jones, 1964), the adult mudsucker becomes quite still in critically low oxygen tensions and ceases all opercular movements for several minutes before gulping at the surface. This characteristic period of adjustment allows the functional-morphological changes necessary for aerial respiration to proceed to completion.

Air-breathing fishes must somehow compensate for their added buoyancy derived from the gas in their respiratory organ. The gulping mudsucker may accommodate this by attaching itself to the bottom with its fused pelvic fins. If it takes a larger than optimal gulp, it often somewhat erratically expels excess air through its gill cover, although the respired bubbles are always released through the mouth. This means of buoyancy adjustment may serve a dual purpose, in that Carter (1957) noted that most fishes occasionally gulp air and expel it through the gill opening simply to flush the gills of clogging substances. The relative volume of the mudsucker swimbladder is significantly less (3.6–4% of its body volume) than that (about 5%) which, according to Jones and Marshall (1953), is necessary for maintenance of hydrostatic equilibrium in most marine teleosts. But the engulfed bubble usually occupies about 1% of the body volume, so that between gulps, fish are almost neutrally buoyant and normally must attach to the bottom. After the bubble has been expelled, however, these benthic fishes tend to sink.

Next, we have shown that a continuous decrease in oxygen and concomitant increase in carbon dioxide in the engulfed bubble is almost directly proportional to the time that it is held. Schöttle (1931) analyzed similar changes in the concentrations of these gases held continuously in the buccopharynx of the mudskippers *Periophthalmus* and *Bolcophthalmus* while they were breathing in "free air." Their active rate of oxygen consumption approximated that determined in the present study for half-grown fish, which consume oxygen at about the same rate whether they are breathing aerially while emergent or gulping, and others presented by Wells (1935) for fish breathing aquatically. The standard rate of oxygen consumption determined by Barlow (1961a) for environmentally adjusted fish breathing pure oxygen in moist chambers is about half the active rate. The volumetrically determined respiratory quotient for mudsuckers, about 0.3, is significantly less than the expected value, which theoretically should be only slightly less than unity. However, this value is reasonable considering the potential loss of carbon dioxide

to the surrounding sea water both directly and by diffusion through the gills, or perhaps, skin. The volumetric relationship of these gases during gulping, therefore, strongly indicates the occurrence of considerable respiratory exchange in the buccopharyngeal cavity.

The usual stimulus for gulping is most likely the presence of critically low dissolved oxygen tensions, though mudsuckers do respond to abnormally high concentrations of carbon dioxide. That ordinary environmental levels of carbon dioxide can generally stimulate breathing in most fishes, however, is uncertain (reviewed by Fry, 1957; Jones, 1964). Carter (1957) summarized earlier studies that indicated internal air-breathing organs in fishes to contain as much as 3% carbon dioxide; this would prevent full oxygenation of ordinary hemoglobin. After an especially long sojourn on the bottom, air-breathing mudsuckers have up to 4.0% carbon dioxide in their buccopharynx. Willmer (1934) and Fish (1956) showed that some air-breathing fishes are less sensitive to carbon dioxide than obligatory aquatic breathers and Carter suggested that most air-breathers may have evolved a hemoglobin relatively insensitive to this gas. Although *G. mirabilis* gulps normally under pure nitrogen, it gasps erratically under an atmosphere of equal parts nitrogen and carbon dioxide. In oxygenated water it is initially unaffected by bubbled nitrogen and gulps in a few hours, only after the oxygen concentration has become critically low. When in sea water aerated with carbon dioxide, however, the fish gulps after a normal period of adjustment and soon develops the "red mouth" typical of air-breathing individuals. When it continues gulping in a small unrenewed volume of air, gulps progressively increase in volume and erratic gasping occurs. In this confinement carbon dioxide may rise to 3% or 4% as the oxygen content decreases. Therefore, we suggest that dissolved carbon dioxide in sufficient quantities may reinforce the effects of depleted oxygen in initiating gulping and that relatively high concentrations in air cause hyperpnea.

The buccopharyngeal epithelium of mudsuckers is obviously specialized for respiratory blood-vascular transport, in that its superficial proliferation of capillaries is greatest between the tongue and buccal roof, where the air bubble is held. As in other air-breathing gobies (and air-breathing fishes in general), this vascularization is simply a modification of homologous systems in non-air-breathing relatives and presents no innovation in general systemic circulation (*cf.* Schöttle, 1931). Mott (1957, p. 95) assumed that because this type of "... accessory respiratory circuit is ... in parallel with systemic circuits," the system is relatively inefficient, in that a parcel of oxygenated blood may never make a complete circuit. Nevertheless, Carter (1957) pointed out that because air contains proportionately much more oxygen than does water, any greater efficiency is obviated in air-breathers.

The extensive morphological preparation for buccopharyngeal respiration may occur during the mudsucker's quiescent period of adjustment before gulping. Not only is the potential blood supply increased during this period, but also, related histological changes are initiated, which corrugate, lubricate, and pit the epithelium. Similar but long-term changes, which can be accelerated by chronic injections of thyroxin, convert the buccopharyngeal epithelium in *Pteriophthalmus* (Harms, 1935). Therefore, to survive this critical period the mudsucker must have an auxiliary oxygen supply.

The apparent absence in most instances of all opercular movement probably

eliminates the possible use of gills during this period, when oxygen tensions are already much less than optimal for aquatic respiration. Cutaneous respiration, as observed in several gobies (*e.g.*, in *Neogobius* by Shulman, 1956), is improbable as the major source, also because of insufficient dissolved oxygen. Noting that some Salton Sea mudsuckers with blood-engorged fins move into shoal, better-oxygenated waters at night, however, Walker *et al.* (1961) suggested that a combination of behavioral and physiological (*i.e.*, cutaneous respiration, especially through the fin membranes) mechanisms might help fish endure low nocturnal oxygen tensions.

It is possible that the mudsucker assumes a naturally induced state of torpor or that it metabolizes anaerobically during the adjustment interval. The crucian carp *Cyprinus carpio* can tolerate anoxia for a considerable period (Blažka, 1958). Blažka found that when it was transferred into deoxygenated water it would rest quietly on the bottom of the tank and continue its opercular movements, albeit not so vigorously as before. At 15–20° C., fish endured anoxia for 2–33 hours, depending on their degree of acclimation to low oxygen tensions; but at 5° C., fish remained quietly in this state for two months "without danger." The species is biochemically adapted to survive anoxia, in that the end-products of anaerobic metabolic metabolism are mostly fats, not noxious lower fatty acids, etc. that accumulate in species (*e.g.*, the trout) metabolically ill-adapted to survive anoxia. Nevertheless, he concluded that at higher temperatures anaerobic metabolism yields insufficient energy, even to sustain the carp for long periods. Beadle (1961) further reviewed the mechanisms and consequences of anaerobic metabolism in animals. Animals such as the carp that have evolved mechanisms of anaerobiosis, however, usually lack elaborate means of aerial respiration such as the buccopharyngeal organ of the mudsucker. Furthermore, unlike the carp which must survive the winter in stagnant, often frozen-over ponds, the mudsucker needs endure anoxia only minutes. Although it, like the carp, rests quietly on the bottom when subjected to anoxia, it is certainly not torpid and can dart away at the slightest provocation. For the mudsucker, a similar and therefore more likely mechanism for surviving the quiescent adjustment interval presumes the tapping of an already available though limited oxygen supply, which, before the initial gulp, could only be obtained from the swimbladder. As blood and tissue oxygen is depleted, swimbladder oxygen would automatically enter by simple diffusion through the permeable membrane of the red gland or oval, thereby rendering a more complex biochemical system unnecessary.

The gas content of the physoclistic swimbladder implies its possible function as a respiratory organ, at least in an auxiliary capacity (*e.g.*, Popta, 1910; Fänge, 1953). Berg and Steen (1965) stated (p. 470) that "The oxygen content of the swimbladder must . . . be taken into consideration when the reactions of fishes to situations of asphyxia are studied." When physoclists are held in poorly oxygenated water, the proportion of oxygen in the bladder decreases while carbon dioxide increases (reviewed in Jones and Marshall, 1953; Jones, 1957). Fänge (1953) further demonstrated that this "deflatory response" is caused by either an environmental oxygen deficiency or an excess of carbon dioxide. Other investigators, however, have concluded that because the extra oxygen removed from the bladder would meet the fish's respiratory requirements for only a few (5–15)

minutes, its respiratory function cannot be important relative to the total amount required for continued metabolic needs (reviewed in Jones and Marshall, 1953; Jones, 1957). Nevertheless, Jones (1957) suggested that shallow-living physoclists may draw on swimbladder oxygen in times of stress, and the general consensus predicts that they could depend on swimbladder oxygen for short respiratory emergencies caused by critically low oxygen and/or abnormally high carbon dioxide tensions.

As in other fishes, the swimbladder oxygen content of *G. mirabilis* decreases in deoxygenated water, usually by more than half preparatory to gulping and by as much as 95% under an atmosphere of pure nitrogen. As in others (cf. Kuhn *et al.*, 1963), newly secreted gas is rich in oxygen and contains appreciable amounts of carbon dioxide, although both gases decrease proportionately to about 25–30% and 2–3% of the swimbladder volume, respectively, when the fish are kept in aerated water. Now, assuming that 60% of this oxygen is normally used as an emergency supply during the pre-gulping quiescent period of standard metabolism, a 30-g. adult could respire for 13 minutes during this period, as compared with 17 minutes, the average for all mudsuckers examined. Therefore, their small but well-developed swimbladder, which probably cannot maintain neutral buoyancy in these bottom-dwelling fish, might supply a large part of the required oxygen during the fish's period of adjustment to aerial respiration.

Preliminary observations of closely related *Gillichthys seta* indicate that it gulps in much the same way as *G. mirabilis*. Only half the size of *G. mirabilis*, the adults of *G. seta* resemble halfgrown of *G. mirabilis*, in that they apparently do not cease all opercular movements preparatory to and during gulping, and more readily substitute surface-breathing for bottom sojourns. The swimbladder of *G. seta* resembles that of *G. mirabilis* and contains respiratory gases in similar proportions. In the Gulf of California, another related goby, *Quietula guaymasiae* (Jenkins and Evermann) gulps surface air more simply. Its opercular movements continue at a rapid beat even in deoxygenated water. While holding the bubble in its buccopharynx, it remains at the surface for extended periods with its snout barely projecting. Its buccopharyngeal vascularization is less profuse than that of *Gillichthys* and because it lacks a swimbladder it has no auxiliary oxygen store.

Barlow (1961a, 1961b) suggested that adult *Gillichthys seta* resembles halfgrown *G. mirabilis* in several morphological and physiological characteristics. Our observations of the gulping response tend to support this. The response of *Quietula guaymasiae* is even more generalized and resembles that in the young of *G. mirabilis*. As implied by Schöttle (1931), gobies may be preadapted to aerial respiration; many have evolved generalized to highly specialized mechanisms in response to individual trends in natural selection and exploitation of "extreme" habitats.

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SUMMARY

1. Like several other estuarine gobies, *G. mirabilis*, which ranges from central California through the Gulf of California, can breathe aerially by means of its heavily vascularized buccopharynx.

2. When environmental dissolved oxygen falls below 2.0 mg./l., the fish ceases all opercular movements during a preliminary period of adjustment, then gulps mouthfuls of air at the surface, usually retires to the bottom for a brief sojourn, and expels the respired air before the next gulp.

3. While gulping in the confinement of an inverted funnel, the fish consume oxygen and excrete carbon dioxide in almost direct proportion with the time that the gulped air bubble is held. The relatively low respiratory quotient (RQ) suggests that considerable carbon dioxide is excreted by the gills and, perhaps, skin, even when the fish is gulping. Respirometric measurements indicate rates of oxygen consumption to be similar for all active air-breathing mudsuckers, whether submergent and gulping or emergent and moist.

4. Its spacious buccopharynx is heavily vascularized, especially between the tongue and buccal roof, where the bubble is usually centered. The proliferation of capillaries in the buccopharyngeal epithelium simply represents an evolutionary expansion of homologous systems present in non-air-breathing relatives, rather than an evolutionary innovation. When fish are placed in water low in oxygen, histological changes are initiated which dilate the epithelial capillaries, engorging them with blood, and which corrugate, lubricate, and pit the epithelium, thereby creating a lung-like surface. Within a few minutes after transfer to deoxygenated water, the mouth is reddened by engorged vessels, although full functional adaptation is attained 10–20 minutes later when the fish begins gulping. An obligatory aquatic breather, *Fundulus parvipinnis*, exhibits no such changes.

5. Because during its pregulping period of adjustment the adult mudsucker ceases all opercular movements, an auxiliary oxygen store most likely supplies oxygen for continued aerobic metabolism. The obvious source in the swimbladder could sustain the fish at a standard rate for all or most of this period and, indeed, the percentage of oxygen here decreases by considerably more than half during this time. After being forcefully emptied, the swimbladder is refilled in 12 hours with gas of high oxygen and carbon dioxide concentrations, which decrease to normal after 48 hours. The relatively small swimbladder cannot create neutral buoyancy for the bottom-dwelling mudsucker unless it is augmented by the volume of an unusually large gulp of air.

6. Therefore, the mudsucker conforms to all necessary and sufficient criteria thus far investigated for air-breathing in fishes. Although relatively sluggish, the aquatic-breathing fish tolerates about the same low oxygen tensions as most other species.

7. The Gobiidae include several air-breathing species, among which the mudsucker is intermediate in specialization for aerial respiration between closely related but relatively unspecialized *Quietula guaymasiae* of the Gulf of California and semi-terrestrial *Periophthalmus schlosseri* of southeastern Asia. Its only congener, *G. seta*, resembles it in gulping habit, but exhibits several juvenile characteristics, such as its continuation of opercular movements while air-breathing.

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